

Application No .09/668,482  
Amendment dated August 11, 2003  
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## LISTING OF CLAIMS

Claims 1 to 82 (previously cancelled)

83. (Currently amended) [An isolated] A microsomal preparation comprising a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, [which] wherein said protein oxidizes [a retinoid] all-trans retinoic acid at the C4-position of the  $\beta$ -ionone ring, said [and encoded by a] nucleic acid molecule comprising a nucleotide sequence that hybridizes under high stringency conditions, wherein high stringency conditions include a wash step of about 0.2 x SSC at 50°C, to a polynucleotide having a nucleotide sequence selected from the group of sequences shown as: SEQ ID NO:3; [a sequence which varies from SEQ ID NO:3 in a coding region due to the degeneracy of the genetic code;] SEQ ID NO:5; [a sequence which varies from SEQ ID NO:5 in a coding region due to the degeneracy of the genetic code;] and SEQ ID NO:31; [a sequence which varies from SEQ ID NO:31 in a coding region due to the degeneracy of the genetic code, or a conservatively substituted amino acid variant of a said protein which oxidizes a retinoid] and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.

Claims 84 to 89 (Cancelled)

90. (Currently amended) [An isolated] A microsomal preparation comprising a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, [which] wherein said protein hydroxylates [a retinoid] all-trans retinoic acid at the C4-position [4-position] of the  $\beta$ -ionone ring, said [and encoded by a] nucleic acid molecule comprising a nucleotide sequence that hybridizes under high stringency

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conditions, wherein high stringency conditions include a wash step of about 0.2 x SSC at 50°C, to a nucleic acid molecule having a nucleotide sequence selected from the group of sequences shown as: SEQ ID NO:3; [a sequence which varies from SEQ ID NO:3 in a coding region due to the degeneracy of the genetic code;] SEQ ID NO:5; [a sequence which varies from SEQ ID NO:5 in a coding region due to the degeneracy of the genetic code;] and SEQ ID NO:31; [a sequence which varies from SEQ ID NO:31 in a coding region due to the degeneracy of the genetic code, or a conservatively substituted amino acid variant of a said protein which hydroxylates a retinoid] and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.

Claims 91 to 112 (Cancelled)

113. (New) The preparation of claim 83, wherein said nucleotide sequence hybridizes under said conditions to SEQ ID NO:3.

114. (New) The preparation of claim 83, wherein said nucleotide sequence hybridizes under said conditions to SEQ ID NO:5.

115. (New) The preparation of claim 83, wherein said nucleotide sequence hybridizes under said conditions to SEQ ID NO:31.

116. (New) The preparation of claim 114, wherein the amino acid sequence identity between the protein and SEQ ID NO:4 is at least about 93 percent.

117. (New) The preparation of claim 116, wherein the protein comprises the amino acid sequence identified as SEQ ID NO:4.

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118. (New) The preparation of claim 113, wherein the protein comprises the amino acid sequence identified as SEQ ID NO:2.

119. (New) The preparation of claim 115, wherein the protein comprises the amino acid sequence identified as SEQ ID NO:32.

120. (New) The preparation of claim 83, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 35 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

121. (New) The preparation of claim 120, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 40 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

122. (New) The preparation of claim 121, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 50 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

123. (New) The preparation of claim 122 wherein the nucleic acid molecule encodes an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

124. (New) The preparation of claim 123, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

125. (New) The preparation of claim 124, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

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126. (New) The preparation of claim 125, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

127. (New) The preparation of claim 126, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

128. (New) The preparation of claim 127, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

129. (New) The preparation of claim 128, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

130. (New) The preparation of claim 83, wherein the protein hydroxylates the C18-position of *all-trans* retinoic acid.

131. (New) The preparation of claim 90, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 35 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

132. (New) The preparation of claim 131, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 40 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

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133. (New) The preparation of claim 132, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 50 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

134. (New) The preparation of claim 133, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

135. (New) The preparation of claim 134, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

136. (New) The preparation of claim 135, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

 137. (New) The preparation of claim 136, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

138. (New) The preparation of claim 137, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

139. (New) The preparation of claim 138, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

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140. (New) The preparation of claim 139, where in the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

141. (New) The preparation of claim 90, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

142. (New) A microsomal preparation comprising a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, wherein said protein oxidizes all-*trans* retinoic acid at the C4-position of the  $\beta$ -ionone ring, said nucleic acid molecule encoding an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32, and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.

143. (New) The preparation of claim 142, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

144. (New) The preparation of claim 143, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

145. (New) The preparation of claim 144, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

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146. (New) The preparation of claim 145, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

147. (New) The preparation of claim 146, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

148. (New) The preparation of claim 147, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

149. (New) The preparation of claim 142, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

 150. (New) A microsomal preparation comprising a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, wherein said protein hydroxylates all-*trans* retinoic acid at the C4-position of the  $\beta$ -ionone ring, said nucleic acid molecule encoding an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32, and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.

151. (New) The preparation of claim 150, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

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152. (New) The preparation of claim 151, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

153. (New) The preparation of claim 152, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

154. (New) The preparation of claim 153, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

155. (New) The preparation of claim 154, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

 156. (New) The preparation of claim 155, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

157. (New) The preparation of claim 150, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

158. (New) The preparation of claim 83, wherein the preparation is enriched at least 6.3 fold in said oxidase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.



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159. (New) The preparation of claim 90, wherein the preparation is enriched at least 7.8 fold in said hydroxylase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

160. (New) The preparation of claim 142, wherein the preparation is enriched at least 6.3 fold in said oxidase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

161. (New) The preparation of claim 150, wherein the preparation is enriched at least 7.8 fold in said hydroxylase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

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